Chitty Lyn (Orcid ID: 0000-0002-4857-7138)
Ghidini Alessandro (Orcid ID: 0000-0002-6662-1630)

In case you missed it: The Prenatal Diagnosis editors bring you the most significant advances of 2019.

Chitty LS[1], Hui L[2], Ghidini A,[3] Levy B,[4] Deprest J,[5] Van Mieghem T,[6] Bianchi DW[7]

Author information:

[1]London North Genomic Laboratory, Great Ormond Street NHS Foundation Trust, and Genetics and Genomic Medicine, UCL Great Ormond Street Institute of Child Health, London, UK.

[2] Department of Obstetrics and Gynaecology, University of Melbourne, Parkville VIC Australia

[3]Antenatal Testing Centre, Inova Alexandria Hospital, Alexandria, VA, USA.

[4]Departments of Pathology and Cell Biology, Columbia University, New York, NY, USA.

[5]Departments of Obstetrics and Gynaecology, University Hospitals Leuven, Leuven, Belgium.

[6]Department of Obstetrics and Gynaecology, Mount Sinai Hospital and University of Toronto, Toronto, Canada.

[7]Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA.

Corresponding author

Lyn S Chitty

l.chitty@ucl.ac.uk

Conflicts of interest and funding:

LSC is partially funded by the NIHR Biomedical Research Centre at Great Ormond Street Hospital. The views expressed in this article are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

INTRODUCTION

This year, the editors of *Prenatal Diagnosis* met on a damp autumn day in rural England to make plans for the Journal's next year (Figure 1). Following our now well-established tradition, we identified the 'hot' topics relevant to prenatal diagnosis that had emerged over the past year to collate into a brief review to help our readers in case you had missed it. This year we cover developments that may change our clinical practice, including fragmentomics (a biological feature of cell free DNA); non-invasive prenatal diagnosis (NIPD) for monogenic disorders; artificial intelligence and machine learning; the prenatal phenotypes emerging with increased use of fetal exome sequencing; recent developments in fetal surgery and the artificial placenta. These are all areas that are set to change the way we deliver prenatal diagnosis and obstetric care. We hope you enjoy the read.

Fragmentomics

The biological phenomenon of circulating cell-free nucleic acids has created enormous opportunities for ground-breaking discoveries in clinical medicine. The field of prenatal diagnosis has been the first to successfully translate cell free DNA (cfDNA) into wide-scale clinical practice with non-invasive prenatal testing (NIPT). We know that the fetal portion of cfDNA in maternal plasma comes from the cytotrophoblast[1] and that, in most circumstances, the maternal haematopoietic cells contribute the majority of the circulating cfDNA[2]. Despite the enormous progress made during the first two decades of cfDNA, a major remaining challenge is how to determine the fetal origin of cfDNA fragments without relying on unique fetal sequences, such as Y-chromosome specific sequences or polymorphisms. Being able to measure the relative proportion of placental and maternal DNA – the so-called fetal fraction (FF) – is now recognised as a crucial quality assurance metric[3,4]. However, laboratories currently use a variety of methods that are not directly comparable, with the Y-

chromosome based method remaining the "gold standard" despite only being applicable to 50% of pregnancies[5]. Another challenge related to the problem of reproducibly measuring FF is the determining the tissue of origin of aberrant cfDNA when a source other than the placenta is suspected, for example where a chaotic plasma genomic profile obtained during non-invasive prenatal testing (NIPT) raises suspicion of a maternal neoplasm[6,7] If a suspected tumoural source of cfDNA could be inferred from the maternal plasma cfDNA, it would improve the management of these pregnant women.

The emerging knowledge base on the fundamental biology of cfDNA has been called "fragmentomics". Compared to maternal cfDNA, cfDNA of fetal origin is shorter[8,9], differentially-methylated[10,11], contains a 'nucleosome footprint' that reflects a non-random fragmentation pattern[12], and has characteristic end sites that differ from maternal fragments[13].

In 2019, pioneering researchers at the Li Ka Shing Institute of Health Sciences consolidated these understandings of fetal cfDNA in a publication that opens further opportunities for prenatal diagnosis, oncology and transplant medicine. In this proof-of-principle study, the authors proposed a new method for nucleosome positioning profiling and quantitative determination of the relative contributions of various tissues in plasma DNA by fragmentation pattern analyses[14]. In this elegant paper, the investigators capitalise on the knowledge that cfDNA fragmentation is not random, but occurs in tissue-specific patterns that reflect open chromatin regions and nucleosome position, and is deducible by sequencing coverage patterns. Fetal and maternal cfDNA fragments also have their own pattern of 'preferred' end sites. This results in differences in the read densities of sequences corresponding to the orientation of the upstream and downstream ends of cfDNA molecules in relation to the reference genome ('orientation aware'). When compared with the "gold standard" Y-

chromosome based method of FF measurement from first trimester plasma samples, the orientation-aware cfDNA fragmentation value (OCF) approach showed strong correlation with FF.

In the next decade, we should expect an acceleration of clinical applications in cfDNA based on the rapid gains in the new field of fragmentomics. This will not be limited to refining the performance of NIPT through better FF measurement, or the occasional individualised analysis of cfDNA samples from women with suspected occult malignancies, but has huge diagnostic potential in organ transplant recipients and cancer patients. The cfDNA revolution that occurred in prenatal screening and diagnosis is thus set to expand to other fields in medicine.

Non-invasive prenatal diagnosis (NIPD)and screening for monogenic disorders

NIPD for monogenic disorders has been an established clinically accredited service in the UK since 2012 for selected conditions in families at known increased risk, either because of a family history or ultrasound findings suggestive of a monogenic disorder.[15] It is less widely used elsewhere in the world, where it is largely delivered on a research basis rather than as an accredited service. This year saw further publications expanding the potential for NIPD for recessive disorders. The first one reported the use of next generation sequencing (NGS) in pregnancies at risk of sickle cell disease.[16] One advantage of the approach described is that it does not require the paternal genotype. However, whilst the sensitivity of this test was 100% in the presence of sufficient FF, performance was less good at lower thresholds. The authors proposed that this test could be used to triage for invasive testing which would only be required to confirm positive results.[16]. The second paper described the clinical implementation of relative haplotype dosage for the definitive NIPD of cystic fibrosis, with an average turnaround time of 5.75 days and a low inconclusive rate. These authors reported excellent performance, but it has the disadvantage of requiring samples from both parents

and an affected child or sibling, as it is based on a linkage approach.[17] Both of these papers described NIPD in pregnancies at known increased risk, however, 2019 saw the publication of two papers describing screening of low risk pregnancies for monogenic disorders.[18,19] These papers have been used to support at least three commercial companies launching platforms publicly available for screening pregnancies at general risk for monogenic disorders.

The first report uses an NGS approach to screen 422 pregnancies for pathogenic or likely pathogenic variants in 30 genes associated with dominant monogenic disorders that can lead to significant adverse outcomes, including skeletal dysplasias, Noonan spectrum disorders, Rett syndrome etc.[18] Positive results were reported in 35 cases, of which 31 had an abnormal ultrasound scan and four a positive paternal history. Follow-up was only available in 147 of the 422 pregnancies tested (34.8%) which confirmed 20 true positives and 127 true negatives.

The second publication also used an NGS-based method for molecular counting to screen for sickle cell disease, cystic fibrosis, spinal muscular atrophy, alpha-thalassemia, and beta-thalassemia.[19] The approach used in this report was to first screen the pregnant mothers' germline DNA, and the cfDNA in maternal plasma is reflex tested in mothers subsequently identified as carriers to determine the fetal genotype. Much of the data reported in this paper comes from modelling experiments using spiked-in genomic DNA, which may be a useful starting point for assay development but, as discussed above, we know that fetal cfDNA is different from maternal cfDNA or postnatal DNA.[14] There appears to be a high inconclusive rate in this study, although the FF was reported at >10% in most cases. Of note, none of the genotypes reported included pathogenic mutations. Furthermore, most of the genes included in this study have multiple pathogenic mutations and it is unclear how many of these were included in the assay described.

These papers published in 2019 clearly indicate that NIPD for monogenic disorders is being extended to both cover more conditions but also to reach out to families at general risk through commercial platforms. The evolution of NIPD in high-risk pregnancies to include more conditions is clearly needed, as many families are at significant risk and we know that both parents and health professionals value safer prenatal diagnosis. However, any extension must be accompanied by robust validation. New dominant mutations occur in around 1 in 600 individuals and account for significant mortality and morbidity, potentially justifying screening in low risk pregnancies. The recessive conditions described in the paper by Tsao and colleagues are all serious, with some amenable to neonatal therapy, and perhaps soon in-utero therapy will be available, again potentially justifying attempts for better screening and diagnosis. However, it is of concern to see commercial tests launched with relatively little validation, including in one instance no pathogenic cases. Furthermore, as we move into screening low risk pregnancies we need to understand what parents want, and indeed where they perceive the limit for testing to be. How can we counsel them appropriately to ensure informed parental decision making? How do we explain the uncertainty that accompanies some diagnoses, and who is going to do the counselling? What is certain is that we are likely to see more publications in 2020 and beyond describing screening for monogenic disorders using analyses of cfDNA. We must hope that commercial companies will adhere to the strict standards required for accredited laboratories around the world, before launching tests that may raise unrealistic expectations and require significant pre- and post-test counselling.

Artificial Intelligence and Machine Learning

Artificial intelligence (AI) and machine learning are emerging technologies that have already had major influences on our daily lives (e.g., personalised news feeds, online shopping algorithms, and

robots judging gymnastic competitions). Deep learning is a sub-category of machine learning; it is based on analyses of actual data and has the capacity to continuously improve its performance with additional data and feedback loops. During 2019, papers began to appear that applied this technology to areas of interest to readers of *Prenatal Diagnosis,* including improving blastocyst selection for transfer,[20,21] analysis of placental volume,[22] analysis of multi-omics to predict risk of preterm delivery,[23] and reducing the time for analysis and reporting of newborn genome sequencing data.[24]

A critical need exists to improve embryo selection in order to increase the chance of a live birth following fertility treatment. Time-lapse video imaging is a new technology that allows embryos to remain in culture under stable temperatures and metabolic conditions while being continuously observed and recorded.[20] In two recent studies, investigators hypothesised that artificial intelligence could be used to objectively analyse tens of thousands of video images, thereby improving the approach to selection of the best quality embryos. In the first, expert embryologists curated a high quality data set of developing embryos that was used to train the system.[20] The trained algorithm could distinguish between good and poor quality embryos with 96.4% accuracy. However, this algorithm could not successfully distinguish between those embryos likely to result in a live birth and those that would not. By contrast, another group performed a retrospective analysis of videos obtained from 10,638 fresh and vitrified embryos to develop a model of predicting pregnancy to the fetal heart beat stage.[21] The diagnostic accuracy of the model, as measured by average area under the curve (AUC), was 0.93. This promising deep learning approach does not take into account traditional ways of assessing embryos, such as morphology grade, biologic or metabolic activity.

However, it has the potential to improve stability of embryo culture, laboratory efficiency and ultimately, rates of healthy live births.

As an organ that is critical for fetal growth and development, it seems that the placenta would be an excellent target for the development of machine learning tools for the early prediction of fetal growth restriction. Looney and colleagues at Oxford used deep learning to develop a fully automated three-dimensional ultrasound (3D-US) segmentation technique to estimate placental volume in the first trimester. The system was used to generate first trimester placental volumes, which correlated with infant birth weights at term. While the estimated detection rates of small for gestational age babies (23%) increased over prior non-automated studies, the system is still not good enough to use as a routine screening tool[22].

Prediction of the risk of preterm birth is another major goal of obstetrics. Short cervical length is currently the best available marker, but it has a low positive predictive value. In a recent study, investigators tested the hypothesis that adding metabolomics, proteomics, cytokine analysis, demographic and clinical data, to transvaginal sonographic measurements demonstrating a cervical length of < 15 mm, would do a better job of predicting those pregnancies at risk for preterm birth.[23] This was a small, proof of principle study which suggested that adding multiple layers of clinical and laboratory data was better than using sonographic data alone. Larger studies are needed to validate these preliminary results.

As prenatal genome-wide sequencing gradually becomes incorporated into clinical care,[25,26,27] practical lessons can be learned from the emerging approaches being used in intensive care units.[24]

In prior paediatric clinical sequencing studies, the mean time from exome sequencing to diagnosis was 16 days. In April, however, a team from Rady Children's Hospital in San Diego incorporated rapid automated DNA sequencing, deep phenotyping from the electronic health record (EHR), and AI to automatically annotate and interpret sequencing results. The program was able to prospectively diagnose clinical conditions in three of seven neonatal intensive care unit patients in a median time of 20 hours; treatment changed in all three as a result of the diagnosis.

Automated genome analysis and interpretation has the potential to increase widespread adoption of prenatal and paediatric genomic medicine, areas in which there are shortages of experts available, and analysis is labour intensive. A major hurdle to be overcome in prenatal medicine will be the gaps in knowledge regarding the fetal clinical presentations of monogenic diseases. Fortunately, with the increased prenatal utilisation of chromosome microarrays and exome sequencing, more cases are being described in the literature that demonstrate new knowledge regarding fetal phenotypes.[26,28,29] This should help to facilitate automated prenatal diagnoses in the future, reducing turnaround time and expanding management options.

Expanding our Knowledge of Fetal Phenotypes: Insights from Prenatal Whole Exome Sequencing Studies

This past year we have seen a significant increase in the number of sequencing reports focused on fetuses with anomalies. In particular, two large prospective cohort studies, one from the USA and one from the UK (the PAGE study), reported on trio whole exome sequencing (WES) in unselected pregnancies with fetal structural abnormalities.[30,31] Both studies showed an overall added diagnostic yield of about 10% with higher yields in fetuses with multisystem anomalies and in selected

anatomical systems, e.g. skeletal.[30,31] Of great interest is the observation that "expanded" phenotypes are being encountered in fetuses who are being diagnosed using next generation sequencing methodologies. Since the clinical descriptions of most genetic diseases derive from assessment of paediatric and adult patients presenting with medical issues, we are only now beginning to appreciate how those same genetic diseases may present in the prenatal period.

For example, Meier and colleagues identified a fetus with a known pathogenic *de novo* variant in *FGFR2*, which is typically associated with Apert syndrome.[32] The fetus presented with agenesis of the corpus callosum and bilateral syndactyly of the hands and feet, but no sign of craniosynostosis, which is a hallmark feature of Apert syndrome. Ferretti *et al*. described a similar situation where a fetus with poor intrauterine growth and a pathogenic missense variant in *NSD1* only demonstrated classic signs of Sotos syndrome at one year of age [33] Similarly, autosomal dominant mutations in *RERE* are associated with a neurodevelopmental disorder with or without anomalies of the brain, eye, or heart. This was only clinically evident in a six-month old child followed up after a prenatal WES finding of a *de novo* frameshift variant in *RERE*.[31] In this case, the only prenatal finding was an isolated nuchal translucency (NT) of 3.5 mm.

In some instances, the fetus may "grow into the phenotype" during the pregnancy as reported by Arora *et al*. who described a fetus with an NT that increased from 2.9 mm at 12 weeks to 7 mm at 17 weeks. [34] Additional abnormalities were noted at 28 weeks and included moderate unilateral ventriculomegaly (14 mm), Dandy-Walker malformation and multiple joint contractures (later shown to be arthrogryposis multiplex congenita). [34] The fetus expired two days post birth and WES revealed an inherited homozygous novel splice site variant in *COG8*, a gene associated with a congenital disorder of glycosylation. Another example of an expanded fetal phenotype comes from De Graer

and colleagues who reported novel features (diaphragmatic eventration and duplication of the distal part of the small bowel) of the *PIK3CA*-Related Overgrowth Spectrum (PROS).[35]

As new fetal phenotypes are reported in association with a variety of novel mutations, we need to ensure that enough evidence exists before causally linking a specific variant with specific fetal anomalies. Such evidence usually stems from functional and animal model studies as well as the enrichment of the variant in unrelated fetuses with concordant phenotypes and the absence of the variant in normal individuals.[32]

Current clinical practice in postnatal patients dictates that, among other things, detailed phenotyping is necessary for accurate interpretation of sequence variants revealed by WES. This presents a major challenge during pregnancy as the anomalies that we expect in paediatric or adult patients may not be apparent (e.g. seizures, intellectual disability) or may not yet have manifested at the gestational period when the fetus is assessed. Furthermore, subtle or different features may be discernible in the prenatal period which may be different to those observed in the postnatal stage.[32] Large scale efforts to generate public databases with well curated fetal phenotypes and their associated WES findings will greatly enhance our ability to provide meaningful WES results to patients identified with fetal anomalies.

Advances in fetal therapy

Fetal therapy contributes significantly to the literature, and this year we highlight maternal issues as well as technological developments that could enhance our ability to deliver in-utero therapy. In 2019 two papers described the potential maternal complications of fetal surgery.[36,37] The first was a systematic review of 166 studies reporting either open or endoscopic in-utero surgery. This showed

that the risk for any maternal complication was higher (20.9%) for open fetal surgery compared to endoscopic surgery (6.2%). When considering only severe complications, the difference was smaller; 4.5% for open and 1.7% for endoscopic surgery.[36] However, in this review around a quarter of studies reviewed were excluded as they failed to comment on maternal outcomes. Of note, this review also showed that open fetal surgery increased the risk of preterm labour in subsequent pregnancies, but not uterine dehiscence or rupture. This is in contrast to the second study that reported maternal outcomes for 77 women undergoing hysterotomy for fetal spina bifida repair. Here they showed that the risk for rupture (9.6%) and perinatal death was similar to that seen after classical caesarean section.[37] This is an important driver in the further development of endoscopic techniques. The other maternal issue being addressed is whether and how human immunodeficiency virus (HIV) or viral hepatitis seropositive mothers can be offered fetal surgery, with regard to the risk of intraoperative viral transmission to the fetus. Shamshiraz and colleagues propose an ethical framework to extend inclusion criteria for fetal surgery to those pregnant patients with HIV and hepatitis B or C infection with low or undetectable viral loads.[38] Moerhrlen and colleagues report the first successful fetal surgery for fetal spina bifida with passive in-utero HBV vaccination.[39]

For complex surgeries, like spina bifida repair, fetoscopy requires amnio-insufflation, but the use of dry carbon dioxide has historically been questioned because it causes fetal acidosis. Recent work demonstrated that this can be mitigated by maternal hyperventilation, along with heating and humidifying the gas.[40,41] Dry CO_2 also has an effect on the fetal membranes but, based on biomechanical studies published this year, heating and humidifying the gas may also reduce the risk for membrane rupture.[42,43]

While the debate continues over whether or not minimally invasive surgery is equally neuroprotective as open surgery and which surgical techniques have the least prematurity risk,[44,45,46] it is worth considering the experience required to complete the learning curve for prenatal spina bifida repair. Centres performing high volumes of in-utero surgery report that it takes up to 30 (open) or 60 (fetoscopy) procedures to become proficient.[47] This raises questions about the viability of smaller programs, and emphasises the need for appropriate high-fidelity training models for this (and other) fetal procedures.[48,49]

Imaging, and increasingly MRI, is key in selecting cases suitable for fetal therapy as it is used for planning, simulating and optimising fetal surgery. An early application is likely to be in the treatment of twin-to-twin transfusion syndrome where a combination of Doppler and MRI are used to determine placenta and vascular structure[50] and, using computer vision and deep learning techniques, the ideal entry point and the laser trajectory can be determined.[51] New methods such as photoacoustic imaging, which can be incorporated into current fetoscopes, can also be used to enhance the identification of placental vessels to improve success of ablation.[52]

Thus, whilst we have seen advances in data collection to demonstrate likely maternal complication rates, more needs to be done in this area to help determine the optimal approaches, including long-term outcome studies. In addition, as we see new technologies being applied we must ensure good, (randomised) prospective trials are performed to obtain objective evidence of the best approaches to use.

The artificial placenta

We have chosen to feature the artificial placenta in this year's 'In case you missed it' as a few important manuscripts describing the methodology and physiology of this technology were published in 2019, bringing it closer to clinical translation. Creating an artificial placental and uterine environment as a life support device for extremely preterm neonates has been an endeavour since the 1960s. It is now two years since the first successful attempts at keeping severely preterm lambs alive for up to 28 days in an artificial uterine environment were reported.[53] In the last year, significant further progress has been made both in demonstrating the physiological effects of the artificial environment, and the feasibility in younger animals.

In 2019, several groups have shown that important components of fetal physiology are maintained on artificial placental support and develop more naturally than in a ventilated newborn. Stable cardiovascular physiology has been shown both in short and long-term experiments using a pumpless system.[54,55] Even after longer term support, animals can be transitioned to normal care[53] and maintain normal behaviour and mitochondrial respiration.[56] Previous experiments with a pump-driven system had already shown that fetal splenic development[57] and cerebral oxygenation can be maintained,[58] and that white matter integrity is preserved with this type of life-support.[59] Even though still limited, these experiments give preliminary support for the safety of the artificial placenta.

Further supporting a translation to the human setting, the group at the Children's Hospital Of Philadelphia (CHOP) has now demonstrated that fetal umbilical catheterisation can also successfully be achieved in significantly younger lambs (85-96 days gestation) than previously reported. In addition, the research group in Perth has confirmed the feasibility of a pumpless system that can be

applied to lambs at 95 days gestation and weights of 600g. This is comparable to the 23-week old human fetus, which would be the clinical target population.[60]

Finally, early evidence with artificial placental systems has increased our understanding of normal fetal physiology, for example, the importance of erythropoietin to support red blood cell development and prevent fetal anaemia (and subsequent need for transfusions).[61] Experiments with chronic hypoxemia in this system have advanced our knowledge of the pathophysiology of this condition.[62]

Over the next five to ten years we expect further dissemination of the artificial placenta system throughout research labs worldwide, providing us with more information on the physiology and pathophysiology of different conditions. Major hurdles still need to be overcome before this technology can translate to clinical care but, if it does, this would be a gigantic leap forward in the care of extremely preterm newborns. Additionally, and of relevance to the readers of Prenatal Diagnosis, the ex-utero fetal environment could become an ideal platform for fetal intervention, as limitations due to preterm birth or trans-maternal access would be easily overcome.

References

1. Alberry M, Maddocks D, Jones M, et al. Free fetal DNA in maternal plasma in anembryonic pregnancies: confirmation that the origin is the trophoblast. Prenatal Diagnosis 2007; 27: 415–418.
2. Lui YY, Chik KW, Chiu RW, et al. Predominant hematopoietic origin of cell-free DNA in plasma and serum after sex-mismatched bone marrow transplantation. Clin Chem 2002; 48: 421–427.

3. Canick JA, Palomaki GE, Kloza EM, et al. The impact of maternal plasma DNA fetal fraction on next generation sequencing tests for common fetal aneuploidies. Prenat Diagn 2013; 33: 667–674.
4. Hui L, Bianchi DW. fetal fraction and noninvasive prenatal testing: what clinicians need to know. Prenat Diagn 2019; dos:10.1002/pd.5620
5. Van Beek DM, Straver R, Weiss MM, et al. Comparing methods for fetal fraction determination and quality control of NIPT samples Prenat Diagn 2017, 37, 769–773.
6. Osborne CM, Hardisty E, Devers P, et al. Discordant noninvasive prenatal testing results in a patient subsequently diagnosed with metastatic disease. Prenat Diagn 2013; 33: 609–611.
7. Dharajiya NG, Namba A, Horiuchi I, et al. Uterine leiomyoma confounding a noninvasive prenatal test result. Prenat Diagn 2015; 35: 1–4.
8. Yu SC, Chan KC, Zheng YW, et al. Size-based molecular diagnostics using plasma DNA for noninvasive prenatal testing. Proc Natl Acad Sci U S A. 2014;111(23):8583-8.
9. Fan HC, Blumenfeld YJ, Chitkara U, et al. Analysis of the size distributions of fetal and maternal cell-free DNA by paired-end sequencing. Clin Chem. 2010; 56(8):1279-86.
10. Nygren AOH, Dean J, Jensen TJ, et al. Quantification of fetal DNA by use of methylation-based DNA discrimination. Clin Chem 2010; 56: 1627– 1635.
11. Sun K, Lun FMF, Leung TY, et al. Noninvasive reconstruction of placental methylome from maternal plasma DNA: Potential for prenatal testing and monitoring. Prenat Diagn. 2018;38(3):196-203.
12. Straver R, Oudejans CB, Sistermans EA, Reinders MJ. Calculating the fetal fraction for noninvasive prenatal testing based on genome-wide nucleosome profiles. Prenat Diagn. 2016;36(7):614-21.

13. Sun K, Jiang P, Wong AIC, et al. Size-tagged preferred ends in maternal plasma DNA shed light on the production mechanism and show utility in noninvasive prenatal testing. Proc Natl Acad Sci 2018; 115: E5106– E5114.
14. Sun K, Jiang P, Cheng SH, et al. Orientation-aware plasma cell-free DNA fragmentation analysis in open chromatin regions informs tissue of origin. Genome Res. 2019. 29: 418-427.
15. Jenkins LA, Deans ZC, Lewis C, Allen S. Delivering an accredited non-invasive prenatal diagnosis service for monogenic disorders and recommendations for best practice. Prenat Diagn 2018;38:44-51.
16. Cutts A, Vavoulis DV, Petrou M, et al. A method for noninvasive prenatal diagnosis of monogenic autosomal recessive disorders. *Blood* 2019;134:1190-1193.
17. Chandler N, Ahlfors H, Drury S, et al. Non-invasive prenatal diagnosis for cystic fibrosis: Implementation, uptake, outcome and implications. Clin Chem 2019; Sep 24. pii: clinchem.2019.305011
18. Zhang J, Li J, Saucier JB, et al. Non-invasive prenatal sequencing for multiple mendelian monogenic disorders using circulating cell-free fetal DNA. Nat Med 2019;25:439-47.
19. Tsao, DS, S Silas, BP Landry, NP Itzep, AB Nguyen, S Greenberg, CK Kanne, VA Sheehan, R Sharma, R Shukla, PN Arora, and O Atay 2019 A novel high-throughput molecular counting method with single base-pair resolution enables accurate single-gene NIPT. *Sci Rep* 9 14382.
20. Khosravi P, Kazemi E, Zhan Q, et al. Deep learning enables robust assessment and selection of human blastocysts after *in vitro* fertilization. *NPJ Digital Medicine* 2019; 2:21; https://doi.org/10.103/s41746-019-0096-y.

21. Tran D, Cooke S, Illingworth PJ, Gardner DK. Deep learning as a predictive tool for fetal heart pregnancy following time-lapse incubation and blastocyst transfer. *Hum Reprod.* 2019; 34: 1011-1018.

22. Looney P, Stevenson GN, Nicolaides KH, et al. Fully automated, real-time 3D ultrasound segmentation to estimate first trimester placental volume using deep learning. *JCI Insight.* 2018; 3(10):e120178. https://doi.org/10.1172/jci.insight.120178

23. Bahado-Singh RO, Sonek J, McKenna D, et al. Artificial intelligence and amniotic fluid multiomics: prediction of perinatal outcome in asymptomatic women with short cervix. *Ultrasound Obstet Gynecol.* 2019;54:110-118.

24. Clark MM, Hildreth A, Batalov S, et al. Diagnosis of genetic diseases in seriously ill children by rapid whole-genome sequencing and automated phenotyping and interpretation. *Sci Transl Med.* 2019; 11: eaat6177.

25. The International Society for Prenatal Diagnosis, The Society for Maternal Fetal Medicine, and the Perinatal Quality Foundation. Joint position statement from the International Society for Prenatal Diagnosis (ISPD), the Society for Maternal Fetal Medicine (SMFM), and the Perinatal Quality Foundation (PQF) on the use of genome-wide sequencing for fetal diagnosis. *Prenat Diagn.* 2018; 38:6-9.

26. Best S, Wou K, Vora N, et al. Promises, pitfalls and practicalities of prenatal whole exome sequencing. *Prenat Diagn.* 2018; 38: 10-19.

27. Abou Tayoun AN, Spinner NB, Rehm HL, et al. Prenatal DNA sequencing: Clinical, counseling, and diagnostic laboratory considerations. *Prenat Diagn.* 2018; 38: 26-32.

28. Gray KJ, Wilkins-Haug LE, Herrig NJ, Vora NL. Fetal phenotypes emerge as genetic technologies become robust. *Prenat Diagn.* 2019; 39: 811-817.

29. Talati AN, Webster CM, Vora NL. Prenatal genetic considerations of congenital abnormalities of the kidney and urinary tract (CAKUT). *Prenat Diagn.* 2019; 39: 679-692.

30. Lord J, McMullan DJ, Eberhardt RY, et al. Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study. *Lancet.* 2019;393(10173):747-757.

31. Petrovski S, Aggarwal V, Giordano JL, et al. Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study. *Lancet.* 2019;393(10173):758-767.

32. Meier N, Bruder E, Lapaire O, et al. Exome sequencing of fetal anomaly syndromes: novel phenotype-genotype discoveries. *Eur J Hum Genet.* 2019;27(5):730-737.

33. Ferretti L, Mellis R, Chitty LS. Update on the use of exome sequencing in the diagnosis of fetal abnormalities. *Eur J Med Genet.* 2019;62(8):103663.

34. Arora V, Puri RD, Bhai P, et al. The first case of antenatal presentation in COG8-congenital disorder of glycosylation with a novel splice site mutation and an extended phenotype. *Am J Med Genet A.* 2019;179(3):480-485.

35. De Graer C, Marangoni M, Romnee S, et al. Novel features of PIK3CA-Related Overgrowth Spectrum: Lesson from an aborted fetus presenting a de novo constitutional PIK3CA mutation. *Eur J Med Genet.* 2019:103775.

36. Sacco A, Van der Veeken L, Bagshaw E, et al. Maternal complications following open and fetoscopic fetal surgery: A systematic review and meta-analysis. Prenat Diagn. 2019;39(4):251-68.

37. Goodnight WH, Bahtiyar O, Bennett KA, et al. Subsequent pregnancy outcomes after open maternal-fetal surgery for myelomeningocele. Am J Obstet Gynecol. 2019;220(5):494 e1- e7.

38. Shamshirsaz AA, Sellner AA, Erfani H, et al. An Ethical Framework for Research in Maternal-Fetal Intervention in the Presence of Maternal Human Immunodeficiency Virus or Hepatitis B and C Infection. Obstet Gynecol. 2019;133(1):48-52.

39. Moehrlen U, Elrod J, Ochsenbein-Kolble N, et al. In utero Hepatitis B Immunization during Fetal Surgery for Spina Bifida. Fetal Diagn Ther. 2019:1-4.

40. Amberg BJ, Hodges RJ, Kashyap AJ, et al. Physiological effects of partial amniotic carbon dioxide insufflation with cold, dry vs heated, humidified gas in a sheep model. Ultrasound Obstet Gynecol. 2019;53:340-7.

41. Baschat AA, Ahn ES, Murphy J, Miller JL. Fetal blood-gas values during fetoscopic myelomeningocele repair performed under carbon dioxide insufflation. Ultrasound Obstet Gynecol. 2018;52:400-2.

42. Bircher K, Merluzzi R, Wahlsten A, et al. Influence of osmolarity and hydration on the tear resistance of the human amniotic membrane. J Biomech. 2019: Oct 16:109419. doi: 10.1016/j.jbiomech.2019.109419

43. Sanz Cortes M, Castro E, Sharhan D, et al. Amniotic membrane and placental histopathological findings after open and fetoscopic prenatal neural tube defect repair. Prenat Diagn. 2019;39:269-279

44. Sanz Cortes M, Davila I, Torres P, et al. Does fetoscopic or open repair for spina bifida affect fetal and postnatal growth? Ultrasound Obstet Gynecol. 2019;53:314-323.

45. Sanz Cortes M, Torres P, Yepez M, et al. Comparison of brain microstructure after prenatal spina bifida repair by either laparotomy assisted fetoscopic or open approach. Ultrasound Obstet Gynecol. 2019. Jun 20. doi: 10.1002/uog.20373

46. Sanz Cortes M, Castro E, Sharhan D, et al. Amniotic membrane and placental histopathological findings after open and fetoscopic prenatal neural tube defect repair. Prenat Diagn. 2019;39:269-79.

47. Joyeux L, De Bie F, Danzer E, et al. Learning curves of open and endoscopic fetal spina bifida closure: a systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2019. Jul 4. doi: 10.1002/uog.20389.

48. Phithakwatchara N, Nawapun K, Viboonchart S, et al. Simulation-based fetal shunting training. Prenat Diagn. 2019. Oct 31. doi: 10.1002/pd.5599

49. Javaux A, Bouget D, Gruijthuijsen C, et al. A mixed-reality surgical trainer with comprehensive sensing for fetal laser minimally invasive surgery. Int J Comput Assist Radiol Surg. 2018;13:1949-57.

50. Torrents-Barrena J, Piella G, Masoller N, et al. Fully automatic 3D reconstruction of the placenta and its peripheral vasculature in intrauterine fetal MRI. Med Image Anal. 2019;54:263-79.

51. Torrents-Barrena J, Lopez-Velazco R, Piella G, et al. TTTS-GPS: Patient-specific preoperative planning and simulation platform for twin-to-twin transfusion syndrome fetal surgery. Comput Methods Programs Biomed. 2019;179:104993.

52. Maneas E, Aughwane R, Huynh N, et al. Photoacoustic imaging of the human placental vasculature. J Biophotonics. 2019:e201900167.

53. Partridge EA, Davey MG, Hornick MA, et al. An extra-uterine system to physiologically support the extreme premature lamb. Nat Commun. 2017 Apr 25;8:15112.

54. Miura Y, Usuda H, Watanabe S, et al. Stable Control of Physiological Parameters, But Not Infection, in Preterm Lambs Maintained on Ex Vivo Uterine Environment Therapy.Artif Organs. 2017;41:959-968

55. Ozawa K, Davey MG, Tian Z, et al. Fetal echocardiographic assessment of the cardiovascular impact of prolonged support in the EXTrauterine Environment for Neonatal Development (EXTEND) system. Ultrasound Obstet Gynecol. 2019 Apr 15. doi: 10.1002/uog.20295.

56. Rossidis AC, Angelin A, Lawrence KM, et al. Premature Lambs Exhibit Normal Mitochondrial Respiration after Long-Term Extrauterine Support. Fetal Diagn Ther. 2019;46:306-312

57. McLeod JS, Church JT, Coughlin MA, et al. Splenic development and injury in premature lambs supported by the artificial placenta. J Pediatr Surg. 2019;54:1147-1152

58. El-Sabbagh AM, Gray BW, Shaffer AW, et al. Cerebral Oxygenation of Premature Lambs Supported by an Artificial Placenta. ASAIO J. 2018;64:552-556.

59. Church JT, Werner NL, Coughlin MA, et al. Effects of an artificial placenta on brain development and injury in premature lambs. J Pediatr Surg. 2018;53:1234-1239.

60. Usuda H, Watanabe S, Saito M. Successful use of an artificial placenta to support extremely preterm ovine fetuses at the border of viability. Am J Obstet Gynecol. 2019;221:69.e1-69.e17.

61. Mejaddam AY, Hornick MA, McGovern PE, et al. Erythropoietin Prevents Anemia and Transfusions in Extremely Premature Lambs Supported by an EXTrauterine Environment for Neonatal Development (EXTEND). Fetal Diagn Ther. 2019;46:231-237.

62. Rossidis AC, Baumgarten HD, Lawrence KM, et al. Chronically Hypoxic Fetal Lambs Supported by an Extra-Uterine Device Exhibit Mitochondrial Dysfunction and Elevations of Hypoxia Inducible Factor 1-Alpha. Fetal Diagn Ther. 2019;45:176-183.

Figure 1.

The Editors in wet, rural England

PD_5632_IMG_4882.JPG